PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: **Harald Sontheimer *et al.***

Application No. **08/980,395**

Examiner: **Sheela Huff**

Filed: **November 28, 1996**

Group Art Unit: **1642**

For: **Novel Method of Diagnosing & Treating Gliomas**

DECLARATION UNDER 37 C.F.R. 1.132

I, Vernon Leon Alvarez do hereby make the following declaration:

1. I have served as Vice President of Research and Development at TransMolecular Inc. since 2001. I have more than twenty-five years of research and development experience in the biomedical and biotechnology industries. From 1999 to 2000, I was Director of Biology at VectraMed Inc. and from 1983 to 1998, I was employed in various positions at Cytogen Corporation, ultimately being promoted to the position of Vice President, Discovery Research. From 1974 to 1983, I held various faculty positions at academic and government institutions in the areas of pathology, cell biology, immunology and biochemistry. I have served as a consultant to the biotechnology and pharmaceutical industries in the area of monoclonal antibodies and monoclonal antibody conjugates for clinical applications, peptides and peptide libraries, phage display libraries, membrane receptors, immunology, tumor vaccines and protein chemistry. In addition, I have published over twenty-five scientific articles and am an inventor on a number of issued patents and patent applications. I earned a B.S. in chemistry from the University of Colorado, and a Ph.D. in physical chemistry from the University of Utah.

2. I have reviewed the Office Action dated September 24, 2001, and in particular the Examiner's questions concerning the ability of chlorotoxin compositions to penetrate

the blood-brain barrier and bind to glioma cells. In response, chlorotoxin does penetrate the blood brain barrier and bind to glioma cells.

3. In *in vivo* experiments, severe combined immunodeficient (SCID) mice bearing xenografted human glioma tumors implanted intracranially were used as an animal model to demonstrate the ability of chlorotoxin to target glioma tumor cells *in vivo*. Animals were given an intracerebral injection of a suspension of $0.5\text{-}1.0 \times 10^6$ D54-MG human glioma tumor cells in the right cerebral hemisphere, as previously described [Soroceanu et al., *Cancer Research*, 58: 4871-4879, 1998], and were followed for 14-17 days, at which time they had developed tumors of at least 5 mm in diameter. These animals then received intravenous injections of ^{125}I-labeled chlorotoxin via the tail vein, and the subsequent ability of chlorotoxin to target the brain tumor was measured after 24 hours. One series of experiments also utilized ^{125}I-labeled EGF as a control, since it is known that the EGF receptor is up-regulated in these tumors.

4. As seen in Table 1, intravenous injection of ^{125}I-labeled chlorotoxin specifically targeted the D54-MG xenografted human glioma tumors implanted in the right hemisphere of the brains of eight of ten animals tested demonstrating that ^{125}I-labeled chlorotoxin crosses the blood brain barrier. The fact that chlorotoxnin crosses the blood brain barrier, and EGF does not, indicates that all of the animals tested had an intact blood brain barrier. Moreover, when chlorotoxin crossed the blood brain barrier, our observations indicated that it concentrated on the right hemisphere of the brain, the glioma tumor being present in the right hemisphere and not the left, indicating the specificity of chlorotoxin binding to the glioma.

5. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements

and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

12/2/01
Date

Vernon Leon Alvarez, Ph.D.

Table 1 – Experimental Data

Treatment	Tumor Implant	Animal Number	% I.D./g tissue (3)		Brain:Blood Ratio		Tumor : Non-Tumor Ratio	Specific Targeting (4)
			Right Brain (1)	Left Brain	Right Brain (1,2)	Left Brain		
^{125}I-Chlorotoxin	D54MG	1	22.11	1.12	3.8	0.2	19.7	+++
		2	8.89	0.41	40.4	1.9	21.7	+++
		3	7.38	0.04	105.4	0.6	184.5	+++
		4	0.32	0.01	6.4	0.2	40.0	+++
		5	10.01	1.54	8.6	1.3	6.5	++
		6	0.35	0.06	1.3	0.2	5.8	++
		7	0.19	0.05	2.7	0.7	3.8	++
		8	0.13	0.02	2.6	0.4	6.5	++
		9	10.82	2.21	0.4	0.1	--	0
		10	4.19	0.55	0.5	0.1	--	0
	None	1	0.54	0.04	0.7	0.0	--	0
		2	0.03	0.06	0.0	0.1	--	0
		3	0.04	0.01	0.1	0.0	--	0
		4	0.29	0.37	0.0	0.1	--	0
		5	0.02	0.02	0.1	0.1	--	0
		6	0.02	0.01	0.1	0.1	--	0
^{125}I-EGF	D54MG	1	0.27	0.09	0.2	0.1	--	0
		2	4.14	1.78	0.1	0.1	--	0
	None	1	1.03	0.87	0.1	0.1	--	0
		2	0.34	0.28	0.1	0.1	--	0

(1) D54-MG tumor cells were implanted in the right hemisphere of the brain.
(2) If the ratio for right brain:blood was less than 1.0, there was no specific targeting.
(3) % I.D./g tissue = percent injected dose per gram of tissue
(4) The Specific Targeting was given a score based on the following system:

Ratio	Score
0-1	0
1-5	+
5-10	++
> 10	+++